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Review

Potential impact of a Moraxella catarrhalis vaccine in COPD

Antonia C. Perez [a,b], Timothy F. Murphy [a,b,c,*]

[a] Clinical and Translational Research Center, University at Buffalo, The State University of New York, 875 Ellicott Street, Buffalo, NY 14203, USA
[b] Division of Infectious Diseases, Department of Medicine, University at Buffalo, The State University of New York, 875 Ellicott Street, Buffalo, NY 14203, USA
[c] Department of Microbiology, University at Buffalo, The State University of New York, 875 Ellicott Street, Buffalo, NY 14203, USA

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ABSTRACT

Moraxella catarrhalis is the second most common cause of exacerbations in adults with COPD, resulting in enormous morbidity and mortality in this clinical setting. Vaccine development for *M. catarrhalis* has lagged behind the other two important causes of exacerbations in COPD, nontypeable *Haemophilus influenzae* and *Streptococcus pneumoniae*. While no licensed vaccine is currently available for *M. catarrhalis*, several promising candidate vaccine antigens have been identified and characterized and are close to entering clinical trials. Key steps that are required to advance vaccines for *M. catarrhalis* along the translational pipeline include standardization of assay systems to assess candidate antigens, identification of a reliable correlate of protection and expansion of partnerships between industry, academia and government to overcome regulatory hurdles. A vaccine to prevent *M. catarrhalis* infections in COPD would have a major impact in reducing morbidity, mortality and healthcare costs in COPD.

Contents

1. Introduction

Chronic lung diseases in which bacterial infection plays an important role in the course and pathogenesis of the disease include chronic obstructive pulmonary disease (COPD), bronchiectasis and cystic fibrosis. In addressing the question of the possible benefit of a vaccine to prevent *M. catarrhalis* infection in these settings, the role that *M. catarrhalis* plays in these settings should be

* Corresponding author at: Clinical and Translational Research Center, University at Buffalo, The State University of New York, 875 Ellicott Street, Buffalo, NY 14203, USA.
E-mail address: murphyt@buffalo.edu (T.F. Murphy).

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considered. Based on studies of the bacteriology of bronchiectasis and cystic fibrosis, *M. catarrhalis* does not appear to play a significant role in these clinical settings [1,2]. On the other hand, *Moraxella catarrhalis* is one of the key bacterial pathogens in the setting of COPD. Preventing *M. catarrhalis* infection in adults with COPD would bring an enormous benefit to these patients. Thus, we will first review COPD and the role that *M. catarrhalis* plays in the course and pathogenesis of the disease to outline the rationale for developing an *M. catarrhalis* vaccine in this clinical setting.

2. Chronic obstructive pulmonary disease

COPD is a debilitating disease of adults. Approximately 65 million people have moderate to severe COPD globally, which is the fourth most common cause of death in the world, and projected to be third by 2030 [3–5]. While death rates from heart disease and stroke are declining, the death rate from COPD has doubled since 1970 [4]. The course of COPD is characterized by intermittent worsening of symptoms called exacerbations [6–10]. Approximately half of exacerbations are caused by bacterial infection [6]. Exacerbations result in enormous morbidity and cost, including doctors office visits, emergency room visits, hospital admissions, respiratory failure and sometimes death. Exacerbations accelerate decline in progressive lung function [11–13] and are the single most important cause of the reduced quality of life experienced by patients with COPD [14–16]. Of patients hospitalized for an exacerbation of COPD, one in five will require re-hospitalization in 30 days. Remarkably, exacerbations of COPD that require hospital admission are associated with a 23% one-year mortality [17,18]. Thus, one of the most urgent areas of new research that is needed to impact patients with COPD is new approaches to preventing exacerbations [19].

3. *Moraxella catarrhalis* has been overlooked as a pathogen in COPD

The four primary bacterial pathogens in COPD are nontypeable *Haemophilus influenzae* (NTHi), *M. catarrhalis, Streptococcus pneumoniae* and *Pseudomonas aeruginosa* [6,20–22] *M. catarrhalis* has been underestimated as a cause of exacerbations of COPD, for three main reasons.

(1) The organism is missed in respiratory cultures because colonies of *M. catarrhalis* (previously called *Neisseria catarrhalis*) resemble commensal *Neisseria*, which are normal flora. In order to detect *M. catarrhalis* in culture, it is necessary to test individual colonies to distinguish them from commensal *Neisseria* and this is simply not done in clinical microbiology laboratories and in most clinical trials of COPD. Thus, *M. catarrhalis* has been misidentified as commensal *Neisseria* in many studies and continues to be overlooked in clinical laboratories worldwide [23].

(2) An extensive literature in the 1950 s through 1980 s emphasized the importance of *S. pneumoniae* and NTHi based on sputum cultures. However, a closer look at the data reveals that *M. catarrhalis* was often detected, but ignored. For example, in the classic article in Lancet in 1953 by May [24], *M. catarrhalis* was detected more often than pneumococcus and NTHi; the author concluded that *M. catarrhalis* (then called *Neisseria catarrhalis*) was an "organism whose pathogenic propensities are known to be slight or nonexistent" and ignored it as a pathogen. Most authors did the same for the next 3 or 4 decades.

(3) The third contributing factor is that few prospective studies of bacterial infection of COPD have been performed in the last 20 years. Most data on the bacteriology of COPD is derived from cultures performed as part of clinical trials to study antibiotics in the treatment of exacerbations of COPD. Such point prevalence data are notoriously inaccurate in estimating disease incidence.

4. Role of *M. catarrhalis* in COPD

A 20-year prospective, observational study of COPD from 1994 to 2014 at the Buffalo VA Medical Center provides one of the few estimates of the burden of bacterial disease based on a longitudinal analysis [22]. Participants underwent monthly clinical evaluations and cultures of sputum using accurate laboratory methods. Using a rigorous definition of the etiology of exacerbation, *M. catarrhalis* caused ~10% of all exacerbations and was the second most common cause of exacerbation of COPD after NTHi, (Fig. 1) [23].

Approximately half of episodes of acquisition of a new strain of *M. catarrhalis* were associated with simultaneous onset of exacerbation (signs of infection) while the other half of acquisitions resulted in no change in baseline symptoms. Strain specific protection develops after acquisition and clearance of a strain [22]. Most patients develop systemic and/or mucosal antibody responses to surface epitopes of their own strain following acquisition and clearance of *M. catarrhalis* [25,26]. Extrapolation of these data indicate that adults with COPD experience 2 to 4 million exacerbations caused by *M. catarrhalis* annually in the US [22]. The most recent editions of the major textbooks of Internal Medicine and Infectious Diseases now recognize *M. catarrhalis* as one of the major causes of exacerbations of COPD [27,28].

There are several *M. catarrhalis* virulence factors that have demonstrated temperature sensitivity, i.e. enhanced expression during cold shock [29,30]. This is interesting considering COPD exacerbations tend to increase in frequency during winter months compared to summer [31–35]. While these studies are limited in number and most do not identify the etiology of these winter exacerbations, the previously mentioned prospective study associated this trend with *M. catarrhalis* specifically. Enhanced viral infections during the winter months may also account in part for the seasonal variation observed with *M. catarrhalis*.

4.1. M. catarrhalis as a co-pathogen with other bacterial species

A growing body of evidence indicates that *M. catarrhalis* often participates as a co-pathogen in respiratory tract infections. In the 20-year prospective study noted above, in sputum samples

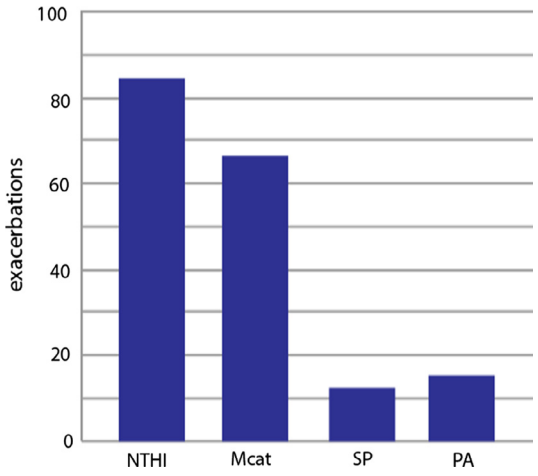

Fig. 1. Cumulative results of a prospective study of bacterial infection in COPD conducted at the Buffalo VA Medical Center from 1994 through 2004. Y-axis: exacerbations as determined by acquisition of a new strain simultaneous with clinical symptoms of an exacerbation. NTHI: nontypeable *H. influenzae*, Mcat: *M. catarrhalis*, SP: *Streptococcus pneumoniae*, PA: *Pseudomonas aeruginosa*.

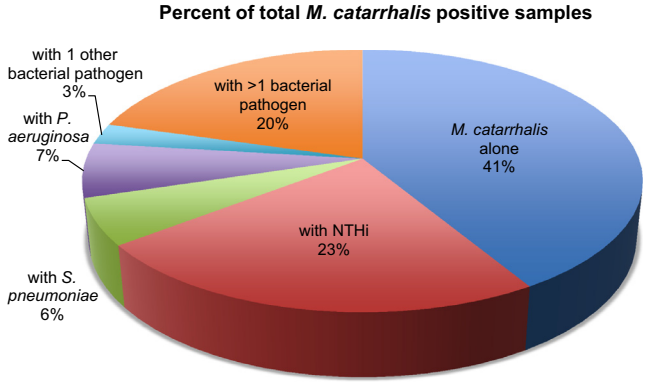

Fig. 2. Cumulative results from prospective COPD studies [22,37,38]. In each study, bacterial species were differentiated by culture or qPCR. Studies using qPCR tend to detect *M. catarrhalis* more frequently in sputum samples than studies that used culture detection alone. Percentages were determined based on the number of culture and/or qPCR positive sputum samples over the total *M. catarrhalis* positive sample. The percentages from each study were then averaged to determine the cumulative results.

from which *M. catarrhalis* was isolated, NTHi was isolated from 26.7%, *S. pneumoniae* was isolated from 5.7%, and *Pseudomonas aeruginosa* was isolated from 4.8% [22]. These data were combined with subsequent prospective studies in Fig. 2, which highlights the role of *M. catarrhalis* as a co-pathogen in COPD [36–38]. Furthermore, *M. catarrhalis* is a co-pathogen in the setting of otitis media in children. Although the focus of this review is on COPD, studies in otitis media reveal important concepts regarding the mechanisms by which *M. catarrhalis* functions as a co-pathogen and will be reviewed briefly.

Co-colonization with *M. catarrhalis* in the nasopharynx increases the risk of acute otitis media when either NTHi or *S. pneumoniae* is present [39]. One important aspect of multispecies infections is the formation of polymicrobial biofilms. Animal models of infection and mucosal biopsies from children with chronic otitis media demonstrate the presence of polymicrobial biofilm formation [40–43], which tend to be more resilient than single species biofilms, enhancing survival of both species by increasing resistance to antimicrobials and host immune defenses. Detection via culture-based methods alone underestimates the frequency of multispecies infections, particularly when biofilms are present. Development of PCR-based detection methods has significantly improved identification of bacterial biofilms, including detection of multiple species biofilms.

Secreted factors, including beta-lactamase, outer membrane vesicles (OMV), and interspecies quorum signal (autoinducer 2 or AI-2), are important virulence mechanisms in multispecies infections with *M. catarrhalis*. These factors act distally and provide a mutually beneficial environment for multiple pathogens to cohabitate in the host. For example, beta-lactamase producing bacteria can provide passive protection to species of bacteria that would otherwise be susceptible to beta-lactam antibiotics. *M. catarrhalis* produces a beta-lactamase that is secreted on OMV. Sensitive strains of NTHi and *S. pneumoniae* are protected from beta-lactam killing when in polymicrobial biofilms with *M. catarrhalis* or in the presence of cell-free supernatants containing OMVs produced by *M. catarrhalis* [40,41,44]. Furthermore, complement resistance factors are also present on *M. catarrhalis* OMVs. Cell-free supernatants containing OMVs protect NTHi from complement-mediated killing *in vitro* [45]. Thus, OMVs produced by *M. catarrhalis* protect susceptible species of bacteria from antimicrobials and host innate immune factors, impairing antibiotic treatment and clearance.

Finally, production of AI-2 is another indirect virulence factor that may promote multispecies infections with *M. catarrhalis*. Although *M. catarrhalis* does not produce AI-2, the bacterium responds to exogenously produced AI-2 through an unidentified mechanism [40,41]. Response to this signal results in increased biofilm formation and enhanced resistance to antimicrobials due to alterations in gene expression. Further studies are needed to more thoroughly characterize the mechanisms mediating interactions via interspecies quorum signaling.

4.2. *M. catarrhalis* as a co-pathogen with viruses

Myriad studies demonstrate a link between several viral pathogens and *M. catarrhalis*, including respiratory syncytial virus (RSV), influenza, and rhinovirus [46–51]. In the past, viral infection was highly underestimated in COPD, mainly because of low sensitivity in conventional culture-based methods. However, recent studies using PCR-based methods have detected viral pathogens in approximately 22–57% of COPD patients during exacerbations. Furthermore, viral-induced exacerbations are often associated with increased severity, duration, and frequency of symptoms, especially in susceptible COPD patients [52–54]. These viral-bacterial co-infections increase the severity of exacerbations in patients with COPD, impairing lung function and prolonging hospitalization [55,52]. While little is known about mechanisms of coinfection, infection with viruses may generate a more favorable environment for bacterial infection and vice versa. For example, pre-incubation of bronchial epithelial cells with *M. catarrhalis* downregulates important mediators in innate antiviral immune responses, allowing enhanced viral infection compared to control cells [49]. Furthermore, a study in juvenile chinchillas demonstrated that intranasal coinfection with RSV and *M. catarrhalis* enhances ascension of *M. catarrhalis* into the middle ears of infected animals [56].

Primary viral infection may also cause an alteration of innate defenses, allowing enhanced colonization and survival of *M. catarrhalis* in the host. Secondary bacterial infections have been well described, and numerous studies in animal models of infection using other common respiratory pathogens support this hypothesis [57–62]; however, studies in *M. catarrhalis* are limited. Further research is needed to assess the effects that respiratory viral infection plays in host susceptibility to *M. catarrhalis* secondary infection.

5. Rationale for *M. catarrhalis* vaccine

Typically, adults and children form antibodies against *M. catarrhalis* antigens following colonization and/or infections during the course of their lives. However, development of sufficient antibody quality and titers (and other yet to be defined host responses) can have a major impact on the level of protection. For the most part, healthy adults have a low carriage rate of *M. catarrhalis*, carry the organism in the nasopharynx for short periods of time, and are protected from *M. catarrhalis* infections [63,64]. On the other hand, young children and adults with COPD carry strains of *M. catarrhalis* for months to years and are more susceptible to infection [22,64,65]. Although a correlate of protection against *M. catarrhalis* has not yet been identified, indirect evidence suggests that antibodies contribute to protection and vaccination could boost antibody titers to protective levels. To this point, adults with COPD develop strain-specific protection following clearance of *M. catarrhalis* [22]. Moreover, adults carry low titer antibodies to oligopeptide permease A (OppA), an important nutritional virulence factor necessary for survival of *M. catarrhalis* in the host and potential vaccine antigen. In the mouse pulmonary clearance model, animals vaccinated with OppA developed high titer antibodies to the protein and showed enhanced pulmonary

Table 1
M. catarrhalis Vaccine Antigens Under Development.

Antigen	Mol mass (kDa)	Putative function	Reference
MID/Hag	200	Adhesin, binds IgD,	[78,99–103]
MchA1, MchA2 MhaB1, MhaB2	184, 201	Adhesin	[91,104,105]
McmA	110	Adhesin	[106]
OppA	~80	SBP of ABC transporter, binds peptides	[107,108,29,109]
Msp 75	~75	Homology to succinic dehydrogenase	[110]
McaP	66	Adhesin and phospholipase	[111,112]
UspA1, UspA2	88; 62 (oligomer)	Adhesins, binds hCEACAM (UspA1), complement, vitronectin, and laminin	[26,79,83,84,89,113]
OMP E	50	Fatty acid transport	[114,115]
OMP CD	45	OMP A–like protein, adhesin, binds mucin	[109,116,117,80,118–121]
CysP	~39	SBP of ABC transporter, binds sulfate	[122]
M35	~35	Porin	[123–125]
SBP2	~30	SBP of ABC transporter, binds arginine	[71,72]
AfeA	~29	SBP of ABC transporter, binds ferric ions	[126]
OMP G1a	~29	Lipoprotein, putative copper transport protein	[127,128]
OMP G1b	~29	Surface molecule	[127,129]
OlpA	24	Homologous with *Neisseria* Opa adhesins	[130,131]
Msp 22	~22	Surface lipoprotein, binds heme	[109,110,121]
PilA	16	Type IV pilus, adhesin, transformation, biofilm	[92,132]
LOS	2.5–4	Detoxified LOS	[81,133–136]
LBP peptide	1.7	Peptide of Lactoferrin binding protein	[96]

clearance following subsequent challenge with *M. catarrhalis* [66]. Several other candidate vaccine antigens induce protective responses in mice (see next section). These studies further support the notion that vaccination could enhance immune responses to protective levels in patient populations at a higher risk of *M. catarrhalis* infections. Thus, vaccination may benefit COPD patients by preventing (1) exacerbations caused by *M. catarrhalis*, (2) colonization of this organism in the upper and lower respiratory tract, and (3) transmission of *M. catarrhalis* between COPD patients and other high risk patient groups.

6. Approaches to *M. catarrhalis* vaccine development

6.1. Characteristics of potential vaccine antigens

Both computational and experimental methods have been used to identify potential vaccine antigens in *M. catarrhalis* (Table 1). In general, ideal candidate vaccine antigens have the following characteristics: (1) exposed on the bacterial cell surface, (2) conserved among isolates, (3) expressed during colonization/infection, (4) immunogenic, and (5) induce protective responses. Potential vaccine antigens in other species of unencapsulated Gram-negative bacteria exhibit these characteristics as well, including NTHi, *N. meningitidis,* and *B. pertussis* [67–70]. The antigens listed in Table 1 exhibit many of these characteristics, and show promise for use in a vaccine against *M. catarrhalis.* For example, SBP2 is one of three substrate-binding proteins (SBP) of an ATP-binding cassette (ABC) transporter system in *M. catarrhalis* [71,72]. In general, SBPs are appealing vaccine targets because of their antigenicity and their role in virulence. SBP2 demonstrates the characteristics noted above and also mediates the uptake of arginine, which is a strict growth requirement for *M. catarrhalis*. Exploiting both the surface exposed location and critical function of the antigen is reminiscent of the strategy employed against NTHi protein D, which is a component of an effective vaccine that is licensed in ~60 countries. Protein D is a surface-exposed glycerophosphodiesterase that mediates the release of phosphorylcholine from host epithelial cells and is also a target of protective antibodies [73].

6.2. Protective responses against M. catarrhalis

A major challenge to the development of an *M. catarrhalis* vaccine is the absence of a reliable correlate(s) of protection. Indirect evidence suggests that the induction of a protective antibody response is an important factor. This hypothesis is mainly based on vaccination studies assessing antigens against NTHi and *S. pneumoniae*; both of which cause similar disease, and, as previously discussed, are often found in coinfections with *M. catarrhalis*. For example, serum bactericidal antibodies are a correlate of protection of NTHi in otitis media and opsonizing antibodies are a correlate of protection for pneumococcal infection [74–77].

Most potential *M. catarrhalis* vaccine antigens were assessed in mice using the mouse pulmonary clearance model (Table 1). Generally, following immunization, high levels of serum antibodies against the target antigen are detected [66,78–80]. In some cases, these antibodies induce opsonophagocytosis and/or serum-mediated killing *in vitro* [81,82]. However, the conditions for bactericidal and opsonophagocytosis assays for *M. catarrhalis* are not well standardized and have yielded varying results among research groups. In part, variability may be due to different conditions in a given assay, necessity for host-specific components (e.g. complement, innate immune cells), the presence of blocking antibody, natural serum resistance by the organism or other factors.

Other potential roles of protective antibodies may involve inhibition of the antigen by interfering with its activity in the host, which include blocking nutrient acquisition or bacterial adhesion [68]. For example, UspA2 mediates both bacterial adherence and serum resistance. This outer membrane protein binds extracellular matrix proteins, including vitronectin and laminin, to aid in adherence [83–85] and evasion of complement-mediated killing [86–88]. Vaccination studies in the mouse pulmonary clearance model have demonstrated that the induction of antibodies against this molecule correlates with protective responses [79,89].

6.3. Animal model systems used for vaccine development

The mouse pulmonary clearance model has been used extensively to assess potential vaccine antigens against *M. catarrhalis*. Pulmonary inoculation is achieved by direct inoculation or aerosol challenge. After several hours, the lungs are harvested, homogenized, and plated to enumerate the remaining bacteria. An enhanced rate of clearance over unvaccinated or vehicle controls indicates development of a potentially protective response(s).

While this model is widely accepted, its main limitation is it does not resemble disease in humans. *M. catarrhalis* is a human-evolved pathogen that does not colonize or persist well in experi-

mental animal models. The duration of lung colonization in a naïve mouse challenged in the pulmonary clearance model is only ~24 h, even when challenged with a high inoculum [90]. On the other hand, this model is well characterized and yields highly reproducible results. Therefore, the model allows for reliable, cost-effective assessment of potential vaccine antigens.

More recently, other model systems have been developed to try to better simulate *M. catarrhalis* infection in humans. Most notably, the chinchilla model for nasopharyngeal colonization has shown promise for assessing potential vaccine antigens against *M. catarrhalis* [91,92]. This model has also been used successfully with NTHi and *S. pneumoniae* to simulate nasopharyngeal colonization and middle ear infection [57,93–95]. However, chinchillas clear *M. catarrhalis* from the middle ear, are not as cost-effective, require special housing accommodations, and USDA-approved breeding facilities are limited. While nasopharyngeal colonization is an important initial step, development of better model systems for respiratory tract infections in COPD is a high priority for advancing vaccine development for *M. catarrhalis*.

6.4. Formulation and routes of administration

The antigens listed in Table 1 have been tested in various formulations with different adjuvants and administration routes. Most of the adjuvants tested are not FDA-approved for use in humans, which could hinder or delay the assessment and adoption of an *M. catarrhalis* vaccine. In addition, several routes of administration have been assessed with promising results, including intranasal immunization. An intranasal formulation is a tantalizing option considering *M. catarrhalis* is a mucosal pathogen. Therefore, intranasal vaccines could have the advantage of inducing a protective mucosal immune response, as demonstrated in mice [66,96–98]. However, few intranasal vaccines are licensed for human use, and the adjuvants used to assess *M. catarrhalis* antigens thus far in intranasal formulations are not FDA-approved. Therefore, it may be advantageous to formulate an *M. catarrhalis* vaccine that uses FDA-approved adjuvants and is administered via intramuscular injection, as the majority of the vaccines used in humans are, to expedite the process of FDA-approval and testing in clinical trials.

7. Challenges and future directions

Although *M. catarrhalis* is a major pathogen in two common human ailments (COPD and otitis media), vaccination against this organism is not yet available. By and large, the lag in vaccine development is due to several key issues that must be addressed in order to advance potential vaccine formulations along the translational pipeline.

Identifying a correlate of protection and standardization of assay systems for *M. catarrhalis* are critical to advance vaccine development. *M. catarrhalis* has been overlooked as a pathogen for decades, and only recently have rigorous studies been conducted to investigate pathogenesis of this organism in the setting of COPD. For this reason, improved assay development will be important to elucidate interactions between *M. catarrhalis*, host immune defenses, and other pathogenic species. Of note, identifying a correlate(s) of protection against *M. catarrhalis* and developing better model systems for studying *M. catarrhalis* in COPD are a high priority to advance the field. Current serum killing assays for *M. catarrhalis* are unreliable for use as a marker for efficacy in Phase I clinical trials. Developing standardized *in vitro* assays to assess serum bactericidal activity, opsonophagocytosis, and other functional host responses should provide a more reliable read out for determining vaccine efficacy in humans.

Additionally, expanding partnerships between pharmaceutical companies, academia, and government will be required to move vaccine antigens toward clinical testing. While studies continue to identify and characterize excellent candidate vaccine antigens, none have yet reached testing in Phase I clinical trials. In order to move these antigens along the translational pipeline, purification optimization, GMP quality antigen production and toxicology studies are needed. Funding options for such development are limited, and will likely require gaining interest from industry partners for further antigen production and testing under FDA-approved standards. However, the risk involved with such development and testing puts a steep burden on pharma. Therefore, building creative partnerships between government, academic programs and industry will be critical to development of an effective vaccine to prevent infections caused by *M. catarrhalis*.

A vaccine against *M. catarrhalis* infection on COPD would have an enormous impact in preventing morbidity, reducing healthcare costs substantially, prolonging the life of people with COPD and improving their quality of life.

8. Conflicts of interest

None.

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